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HARRIET M. STRIMPEL, D. Phil.
New England Biolabs, Inc.
240 COUNTY ROAD
IPSWICH, MA 01938-2723

| EXAMINER | |
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| RAMIREZ, DELIA M | |
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**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

STRIMPEL@NEB.COM
Goldberg@neb.com
wermuth@neb.com

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 10/585,964 | GUAN ET AL. | |
| | Examiner | Art Unit | |
| | DELIA M. RAMIREZ | 1652 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *05 November 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1-31* is/are pending in the application.

4a) Of the above claim(s) *18-23,25,29 and 30* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-17,24,26-28 and 31* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *13 July 2006* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *7/13/06,8/2/07*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☒ Other: *alignment*.

## DETAILED ACTION

### *Status of the Application*

Claims 1-31 are pending.

Applicant's election of Group I, claims 1-17, 24 in part, 26-28 and 31, drawn to a modified DNA cleaving enzyme having 35% sequence identity with T7 Endo I, a kit comprising said enzyme, a method of use, and a method of manufacture of said enzyme, as submitted in a communication filed on 11/5/2008 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

Claims 18-23, 25, 29-30 are withdrawn from further consideration by the Examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention. Claim 24 is directed in part to non-elected inventions. Claims 1-17, 24 as it relates to the elected enzyme, 26-28 and 31 are at issue and are being examined herein.

### *Specification*

1. The specification is objected to for the following reasons. Several inconsistencies have been found throughout the specification regarding the use of sequence identifiers. For example, in Figure 11C, the drawing refers to SEQ ID NO: 24 as that of an oligonucleotide, however SEQ ID NO: 24 shows in the sequence listing as an amino acid sequence. Also, the same sequence identifier, i.e., SEQ ID NO: 13, is used for a *Yersinia pestis* phage phiA112 protein (Figure 14-3) and an Enterobacteria phage T7 protein (Figure 13). In addition, a cursory review of SEQ ID NO: 13 in the sequence listing shows that what is being indicated as SEQ ID NO: 13 in these figures is not the same as what is shown in the sequence listing. See, for example, the C-terminus of SEQ ID NO: 13 in the sequence listing (QAKGGKK) and in Figure 13 (KRKGGKK). Applicant is requested to carefully review the application and correct any inconsistencies between the sequence displayed and the sequence identifier used without introducing new

matter into the disclosure. Because it is unclear from the specification which is the actual amino acid sequence identifier for the T7 Endo I protein used in the Examples described in the specification, the examiner has assumed that the wild-type sequence is either SEQ ID NO: 24 or SEQ ID NO: 25 for examination/search purposes.

***Priority***

2. This application is the US National stage of PCT/US04/39288 filed on 11/22/2004.

***Information Disclosure Statement***

3. The information disclosure statements (IDS) submitted on 7/13/2006 and 8/2/2007 are acknowledged. The submissions are in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statements are being considered by the examiner.

***Drawings***

4. The drawings submitted on 7/13/2006 have been reviewed and are accepted by the Examiner for examination purposes.

***Claim Objections***

5. Claims 2-17 are objected to due to the recitation of "A modified....according to claim X..". The term should be amended to recite "The modified....according to claim X" in view of the fact that the modified DNA cleaving enzyme has been defined in claim X. Appropriate correction is required.

6. Claim 4 is objected to due to the recitation of "wherein the product of the altered enzyme activity is a DNA duplex with a single strand over-hang..". To enhance clarity, it is suggested the term be

amended to recite, for example, "wherein the product of the reaction catalyzed by the modified DNA cleaving enzyme is a DNA duplex….". Appropriate correction is required.

7. Claim 7 is objected to due to the recitation of "An A". This appears to be a typographical error. Appropriate correction is required.

8. Claim 9 is objected to due to the recitation of "wherein the altered enzyme activity occurs in magnesium buffer". To enhance clarity, it is suggested the term be amended to recite "wherein the altered enzyme activity is observed in a magnesium-containing buffer". Appropriate correction is required.

9. Claim 11 is objected to due to the recitation of "activity for cleavage a DNA under…". To enhance clarity, the term should be amended to recite, for example, "activity for DNA cleavage..". Appropriate correction is required.

10. Claim 15 is objected to due to the recitation of "wherein the at least one mutation … is a substitution .. or deletion such that the substitution …. is selected from a single amino acid, a dipeptide, a tripeptide, and a tetrapeptide.". To enhance clarity, it is suggested the term be amended to recite "wherein the at least one mutation at the Pro-Ala (PA can also be used) dipeptide is a substitution or a deletion, and wherein said substitution is selected from the group consisting of a single amino acid substitution, a dipeptide substitution, a tripeptide substitution….". Appropriate correction is required.

11. Claim 16 is objected to due the recitation of "PA/A, PA/AA....and PA/P". To enhance clarity, it is suggested the term be amended to recite, for example, "substitution of the Pro-Ala (or PA) dipeptide with amino acid A, substitution of the Pro-Ala (or PA) dipeptide with the Ala-Ala (or AA) dipeptide, substitution of the Pro-Ala (or PA) dipeptide with the Pro-Gly-Ala (or PGA) tripeptide ….deletion of the Pro-Ala (or PA) dipeptide,.....and substitution of the Pro-Ala (or PA) dipeptide with amino acid P". Appropriate correction is required.

12. Claim 24 is objected to due to the recitation of "a modified...of claim 1". The term should be amended to recite "the modified.... of claim 1" since the DNA cleaving enzyme has been defined in claim 1. Appropriate correction is required.

13. Claim 24 is objected to as being dependent upon non-elected claims and also as being directed in part to non-elected claims. Examination of the instant claim will be limited to the elected invention. Appropriate correction is required.

14. Claim 26 is objected to due to the recitation of "DNA substrate with a modified DNA cleaving enzyme according to claim 1". The term should be amended to recite "DNA substrate with the modified DNA cleaving enzyme according to claim 1" since the DNA cleaving enzyme has been defined in claim 1. Appropriate correction is required.

15. Claims 27-28 are objected to due to the recitation of "A method....according to claim X..". The term should be amended to recite "The method....according to claim X" in view of the fact that the method has been defined in claim X. Appropriate correction is required.

16. Claim 31 is objected to as being dependent upon a non-elected claim, i.e., claim 23. For examination purposes, the Examiner will interpret the claim to be an independent claim which includes the limitations of claim 23. Appropriate correction is required.

17. Claim 31 is objected to due to the recitation of "a method for over-expressing T7 ....comprising....and over-expressing the T7 endonuclease I". To be consistent with commonly used claim language, it is suggested the term be amended to recite, for example, "a method for recombinantly producing .....comprising culturing a host cell.....and producing the .....". Appropriate correction is required.

***Claim Rejections - 35 USC § 112, Second Paragraph***

18. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

19. Claims 1-17, 24, 26-28, 31 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

20. Claim 1 (claims 2-17, 24, 26-28, 31 dependent thereon) is indefinite in the recitation of "at least 35% amino acid sequence identity with T7 Endo I" because the term is unclear in the absence of a recitation of the specific sequence with which the comparison should be made. Also, T7 Endo I is not a sequence but a protein. For search purposes, the Examiner has interpreted the term "T7 Endo I" as " SEQ ID NO: 24 or SEQ ID NO: 25". Correction is required.

21. Claims 1, 5, 7 are indefinite in the recitation of "A modified DNA cleaving enzyme… enzyme cleavage activity" for the following reasons. The preamble of the claim indicates that the enzyme's activity is that of cleaving DNA. The term "enzyme cleavage activity" implies that the enzymatic activity is that of cleaving an enzyme (e.g., a protease). For examination purpose, it will be assumed that the term reads "A modified DNA cleaving enzyme… enzyme's DNA cleavage activity". Correction is required.

22. Claim 2 is indefinite in the recitation of "enzyme according to claim 1…having reduced toxicity in a host cell permitting over-expression of the DNA….". As written, there is no basis for comparison regarding the toxicity in a host cell (i.e., reduced toxicity in a host cell compared to what?). For examination purposes, it will be assumed that the claim recites "enzyme according to claim 1…having reduced toxicity in a host cell compared to the toxicity of the unmodified enzyme in the same host cell, wherein said reduced toxicity allows the modified DNA cleaving enzyme to be recombinantly produced in the host cell." Correction is required.

23. Claim 7 is indefinite in the recitation of "wherein an alteration in the enzyme cleavage activity of the modified enzyme compared to the unmodified enzyme occurs in a manganese-containing buffer" for the following reasons. As written it appears as if both the modified and the unmodified enzyme have an

alteration/characteristic which is only evident when the modified enzyme is in a manganese-containing buffer, which is unclear because the unmodified enzyme should not have any alterations. For examination purposes, it will be assumed that the term reads "wherein the DNA cleaving activity of the modified enzyme in a manganese-containing buffer is higher than the DNA cleaving activity of the unmodified enzyme in a manganese-containing buffer". Correction is required.

24. Claim 8 is indefinite in the recitation of "wherein the altered enzyme activity is selected from: maintenance of cleavage activity" because it is unclear as to how maintaining cleavage activity is a form of alteration of enzymatic activity wherein alteration implies change. For examination purposes, it will be assumed that the term reads "increase or decrease in cleavage activity". Correction is required.

25. Claim 10 is indefinite in the recitation of "a reduced ratio of non-specific nuclease activity; and reduction…" because the denominator in the reduced ratio is missing (i.e., reduced ratio of non-specific nuclease activity to what?). For examination purposes, no patentable weight will be given to the term. Correction is required.

26. Claim 11 (claim 12 dependent thereon) is indefinite in the recitation of "having enhanced or reduced activity for cleavage a DNA under modified reaction conditions when compared with the unmodified enzyme" for the following reasons. The term "modified reaction conditions" is unclear because one cannot determine which conditions are being referred to. Correction is required.

27. Claim 12 is indefinite in the recitation of "a modified…according to claim 12 wherein the modification in reaction conditions are selected from changing at least one of…." for the following reasons. First, the claim depends upon itself. In addition, the term "wherein the modification in reaction conditions....." is unclear and confusing. Is the term intended to mean "wherein at least one of the reaction conditions selected from the group consisting of pH, temperature, manganese concentration, magnesium concentration, and reaction time is modified?. Correction is required.

28. Claim 13 is indefinite in the recitation of "selected from a class of enzymes comprising gene 3...., and Roseophage S101 RP endonuclease I" for the following reasons. The recited enzymes appear to be naturally-occurring wild-type enzymes. Therefore, it is unclear if the scope of modified enzymes encompasses also naturally-occurring enzymes or if the claim is intended to limit the genus of unmodified enzymes to those recited in the claim. For examination purposes, the examiner will interpret the claim to be directed to the recited wild-type enzymes as well as variants of the recited wild-type enzymes. Correction is required.

29. Claim 14 (claims 15-17 dependent thereon) is indefinite in the recitation of "wherein the at least one mutation is a mutation at a PA site in the β-bridge" because it is unclear as to what a PA site is. For examination purposes, it will be assumed that the term reads "wherein the at least one mutation is a mutation at a Pro-Ala dipeptide within the β-bridge". Correction is required.

30. Claim 17 is indefinite in the recitation of "wherein the PA dipeptide is located at position 46 and 47 in SEQ ID NO: 13" for the following reasons. First, positions 46-47 of SEQ ID NO: 13 correspond to Val-Pro and not Pro-Ala. In addition, as written, it is unclear if the term is intended to limit the unmodified enzyme to that of SEQ ID NO: 13, or if the term is merely indicating that the Pro-Ala dipeptide is one which corresponds to the dipeptide found in positions 46-47 of SEQ ID NO: 13. For examination purposes, no patentable weight will be given to the term. Claim 17 will be considered a duplicate of claim 15. Correction is required.

### *Claim Rejections - 35 USC § 112, First Paragraph*

31. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

32. Claims 1-17, 24, 26-28, 31 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 1-17 and 24 are directed in part to a genus of DNA cleaving enzymes having at least 35% sequence identity to the polypeptide of SEQ ID NO: 24 or SEQ ID NO: 25, wherein said enzymes have two catalytic centers separated by a β-bridge, and wherein said enzymes are enzymatically active and not sufficiently toxic to kill a host cell, wherein said enzymes have increased enzyme specificity, different activity in the presence of magnesium or manganese, an increased ratio of nicking of a cruciform structure relative to double strand cleavage, an increased ratio of cleaved cruciform DNA to non-cleaved DNA, and/or reduction in nicking opposite a preexisting nick site. It should be noted that the scope of claim 1 and dependent claims do not appear to be limited to mutants of naturally-occurring enzymes as evidenced by claim 13. See Claim Rejections under 35 USC 112, second paragraph, for claim interpretation and discussion of scope, particularly the reasons as to why the Examiner is using SEQ ID NO: 24 and 25 in her interpretation of the claims. Claims 26-28 are directed to a method for determining whether a DNA substrate has a single nucleotide polymorphism wherein said method uses the genus of DNA cleaving enzymes of claim 1. Claim 31 is directed to a method to recombinantly produce the genus of DNA cleaving enzymes of claim 1.

In *University of California v. Eli Lilly & Co.*, 43 USPQ2d 1938, the Court of Appeals for the Federal Circuit has held that "A written description of an invention involving a chemical genus, like a description of a chemical species, 'requires a precise definition, such as by structure, formula, [or] chemical name,' of the claimed subject matter sufficient to distinguish it from other materials". As indicated in MPEP § 2163, the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice,

reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show that Applicant was in possession of the claimed genus. In addition, MPEP § 2163 states that a representative number of species means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus.

While the specification in the instant application discloses the structure of two species of the recited genus of proteins having the desired DNA cleaving activity (i.e., ME(ΔPA) and ME(PA/A)), it provides no clue as to the essential structural elements required in any protein having the required activity and 35 % sequence identity to SEQ ID NO: 24/25. Furthermore, there is no teaching in the specification or the art as to a correlation between structure and the desired DNA cleaving activity.

The claim encompasses a large genus of proteins sharing a limited amount of structural features. A sufficient written description of a genus of polypeptides may be achieved by a recitation of a representative number of polypeptides defined by their amino acid sequence or a recitation of structural features common to members of the genus, which features constitute a substantial portion of the genus. However, in the instant case, the interpreted structural feature, i.e., "35% sequence identity to SEQ ID NO: 24/25", is not representative of all the members of the genus of proteins recited since there is no information as to which are the remaining structural elements required in the recited polypeptides in addition to those recited in the claims such that the desired DNA cleaving activity is displayed, or a correlation between structure and function which would provide those unknown structural features. In addition, while one could argue that the mutants disclosed are representative of the structure of all the members of the genus, such that the recited genus of polypeptides is adequately described by the disclosure of the structure of these mutants, it is noted that the art teaches several examples of how even

small changes in structure can lead to changes in activity. For example, Witkowski et al. (Biochemistry 38:11643-11650, 1999) teach that one conservative amino acid substitution transforms a β-ketoacyl synthase into a malonyl decarboxylase and completely eliminates β-ketoacyl synthase activity. Seffernick et al. (J. Bacteriol. 183(8):2405-2410, 2001) teach that two naturally occurring *Pseudomonas* enzymes having 98% amino acid sequence identity catalyze two different reactions: deamination and dehalogenation, therefore having different function. Therefore, since minor structural changes to a polypeptide may result in changes affecting function, and no additional information correlating structure with the desired activity has been provided, one cannot reasonably conclude that in an unpredictable art such as that of the claimed invention, two species are representative of the structure of the entire genus of proteins having the recited DNA cleaving activity as required by the claims.

Due to the fact that the specification only discloses two species of the genus, and the lack of description of any additional species by any relevant, identifying characteristics or properties, one of skill in the art would not recognize from the disclosure that Applicant was in possession of the claimed invention.

33. Claims 1-17, 24, 26-28, 31 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for (A) variants of the single T7 endonuclease I used in Example 1 wherein said variants differ from that single T7 endonuclease I only by (1) the deletion of a Pro-Ala dipeptide in the β-bridge of that single T7 endonuclease I, or (2) by substituting a Pro-Ala dipeptide in the β-bridge of that single T7 endonuclease I with an alanine residue, (B) a kit comprising said variants, (C) a method to recombinantly produce said variants, and (D) a method to determine whether a DNA substrate has a single nucleotide polymorphism wherein said method requires the variants of (A), does not reasonably provide enablement for (1) any protein having at least 35% sequence identity to a T7 Endonuclease I/ SEQ ID NO: 24/25, wherein said protein has two catalytic domains linked by a β-bridge,

wherein said protein is enzymatically active and not sufficiently toxic to kill a host cell, wherein said protein has increased enzyme specificity, different activity in the presence of magnesium or manganese, an increased ratio of nicking a cruciform structure relative to double strand cleavage, an increased ratio of cleaved cruciform DNA to non-cleaved DNA, and/or reduction in nicking opposite a preexisting nick site, (2) a kit comprising said protein, (3) a method to recombinantly produce said protein, or (4) a method to determine whether a DNA substrate has a single nucleotide polymorphism wherein said method uses that protein. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims. Factors to be considered in determining whether undue experimentation is required are summarized in *In re Wands* (858 F.2d 731, 737, 8 USPQ2nd 1400 (Fed. Cir. 1988)) as follows: 1) quantity of experimentation necessary, 2) the amount of direction or guidance presented, 3) the presence and absence of working examples, 4) the nature of the invention, 5) the state of prior art, 6) the relative skill of those in the art, 7) the predictability or unpredictability of the art, and 8) the breath of the claims. The factors which have lead the Examiner to conclude that the specification fails to teach how to make and/or use the claimed invention without undue experimentation, are addressed in detail below.

***The breath of the claims.*** Claims 1-17, 24, 26-28, 31 are so broad as to encompass (1) any protein having at least 35% sequence identity to a T7 Endonuclease I/ SEQ ID NO: 24/25, wherein said protein has two catalytic domains linked by a β-bridge, wherein said protein is enzymatically active and not sufficiently toxic to kill a host cell, wherein said protein has increased enzyme specificity, different activity in the presence of magnesium or manganese, an increased ratio of nicking a cruciform structure relative to double strand cleavage, an increased ratio of cleaved cruciform DNA to non-cleaved DNA, and/or reduction in nicking opposite a preexisting nick site, (2) a kit comprising said protein, (3) a method to recombinantly produce said protein, or (4) a method to determine whether a DNA substrate has a single nucleotide polymorphism wherein said method uses that protein. The enablement provided is not

commensurate in scope with the claim due to the extremely large number of proteins of unknown structure encompassed by the claims. In the instant case, the specification enables two variants of the single T7 endonuclease I described in Example 1, wherein said variants differ from that single T7 endonuclease I only by (1) the deletion of a Pro-Ala dipeptide in the β-bridge of that single T7 endonuclease I (ME(ΔPA)), or (2) by substituting a Pro-Ala dipeptide in the β-bridge of that single T7 endonuclease I with an alanine residue (ME(PA/A)).

***The amount of direction or guidance presented and the existence of working examples.*** The specification discloses the structure of two single mutants as working examples wherein said mutants have the recited DNA cleavage activity. However, the specification fails to provide any clue as to the structural elements required in any protein having the desired DNA cleavage activity, or a correlation between structure and function which would provide the additional structural features beyond those which correspond to a 35 % sequence identity variant as interpreted. There is no information or guidance as to which amino acid residues in the polypeptides of SEQ ID NO: 24/25 (assuming these sequences correspond to those of a wild-type T7 Endo I) can be modified and which ones are to be conserved to create a variant displaying the functional characteristics required by the claims.

***The state of prior art, the relative skill of those in the art, and the predictability or unpredictability of the art.*** The amino acid sequence of a polypeptide determines its structural and functional properties. While the art discloses several proteins having DNA cleaving activity, neither the specification nor the art provide a correlation between structure and activity such that one of skill in the art can envision the structure of any protein having a DNA cleaving activity as required. In addition, the art does not provide any teaching or guidance as to (1) which changes can be made to the proteins of SEQ ID NO: 24/25 such that the resulting variant would display the desired DNA cleaving activity, or (2) the general tolerance of DNA cleaving enzymes to structural modifications and the extent of such tolerance. It is also noted that neither the specification nor the art provide any teaching as to whether

any DNA cleaving enzyme having the recited % identity and the recited configuration (i.e., 2 separate catalytic domains linked by a β-bridge) can be mutated at the β-bridge and obtain mutants having the desired functional characteristics, i.e., reduced toxicity, increased enzyme specificity, different activity in the presence of magnesium or manganese, an increased ratio of nicking a cruciform structure relative to double strand cleavage, an increased ratio of cleaved cruciform DNA to non-cleaved DNA, and/or reduction in nicking opposite a preexisting nick site.

The art clearly teaches that modification of a protein's amino acid sequence to obtain the desired activity without any guidance/knowledge as to which amino acids in a protein are tolerant of modification and which ones are conserved is highly unpredictable. At the time of the invention there was a high level of unpredictability associated with altering a polypeptide sequence with an expectation that the polypeptide will maintain the desired activity. For example, Branden et al. (Introduction to Protein Structure, Garland Publishing Inc., New York, page 247) teach that (1) protein engineers are frequently surprised by the range of effects caused by single mutations that they hoped would change only one specific and simple property in enzymes, (2) the often surprising results obtained by experiments where single mutations are made reveal how little is known about the rules of protein stability, and (3) the difficulties in designing *de novo* stable proteins with specific functions. The teachings of Branden et al. are further supported by the teachings of Witkowski et al. (Biochemistry 38:11643-11650, 1999) and Seffernick et al. (J. Bacteriol. 183(8):2405-2410, 2001) already discussed above, where it is shown that even small amino acid changes result in enzymatic activity changes.

***The quantity of experimentation required to practice the claimed invention based on the teachings of the specification.*** While methods of generating or isolating variants of a polypeptide were known in the art at the time of the invention, it was not routine in the art to screen by a trial and error process for any number of variants of the polypeptides of SEQ ID NO: 24/25 and determine which ones have the desired DNA cleaving activity. Instead, one of skill in the art would require some knowledge or

guidance as to how structure correlates with function to select those structural variants most likely to have the desired activity so that a reasonable number of species can be selected for testing. In the absence of (1) a rational and predictable scheme for modifying any residue in the polypeptides of SEQ ID NO: 24/25 and obtain a structural variant as recited such that the resulting variant would have the desired DNA cleaving activity, (2) some guidance as to which wild-type DNA cleaving enzymes having the recited % identity and configuration (2 catalytic domains linked by a β-bridge) can be mutated at the bridge to obtain mutants having the desired functional modification, and/or (3) a correlation between structure and activity, one of skill in the art would have to test an essentially infinite number of proteins within the genus of variants having at least 35% sequence identity to SEQ ID NO: 24/25 to determine which ones have the desired activity.

Therefore, taking into consideration the extremely broad scope of the claim, the lack of guidance, the amount of information provided, the lack of knowledge about a correlation between structure and the desired function, and the high degree of unpredictability of the prior art in regard to structural changes and their effect on function, one of ordinary skill in the art would have to go through the burden of undue experimentation in order to practice the claimed invention. Thus, Applicant has not provided sufficient guidance to enable one of ordinary skill in the art to make and use the invention in a manner reasonably correlated with the scope of the claims.

***Claim Rejections - 35 USC § 102***

34. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless -
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

35. Claims 1, 13, 14, 15, 17 are rejected under 35 U.S.C. 102(b) as being anticipated by Beck et al. (UniProt accession number P20314, February 1, 1991). Claims 1, 13-15 and 17 are directed in part to a protein having DNA cleaving activity wherein said protein is a variant of the polypeptide of SEQ ID NO: 24 or 25 having at least 35 % sequence identical to the polypeptide of SEQ ID NO: 24 or SEQ ID NO: 25, has two catalytic centers linked by a β-bridge, wherein said protein is a phage T3 endodeoxyribonuclease, and wherein the variant differs from the polypeptide of SEQ ID NO: 24 or SEQ ID NO: 25 also by a modification at a Pro-Ala dipeptide in the β-bridge of the polypeptide of SEQ ID NO: 24 or 25, wherein said modification is a substitution of the Pro-Ala peptide with another peptide, including a dipeptide. See Claim Rejections under 35 USC 112 first and second paragraphs for claim interpretation and discussion of scope. Beck et al. teach a phage T3 endodeoxyribonuclease which has two catalytic sites linked by a β-bridge wherein said endodeoxyribonuclease is 75.8 % sequence identical to the polypeptide of SEQ ID NO: 25 and 78.5% sequence identical to the polypeptide of SEQ ID NO: 24. See attached alignments. The polypeptide of Beck et al. has a Pro-Glu dipeptide instead of a Pro-Ala dipeptide at the β-bridge. Therefore, the polypeptide of Beck et al. anticipates the instant claims as written.

***Claim Rejections - 35 USC § 103***

36. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

37. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary.

Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

38. Claims 24 and 31 rejected under 35 U.S.C. 103(a) as being unpatentable over Beck et al. (UniProt accession number P20314, February 1, 1991). The teachings of Beck et al. have been discussed above. Beck et al. do not teach a kit comprising the enzyme or a method to recombinantly produce the enzyme.

Claim 24 is directed in part to a kit comprising the polypeptide of claim 1. Claim 31 is directed in part to a method to recombinantly produce the polypeptide of claim 1. See Claim Rejections under 35 USC 112, second paragraph, for claim interpretation.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to (1) make a kit which comprises the enzyme of Beck et al., a DNA substrate to test its activity, and the necessary reagents to carry out an enzymatic assay, and (2) recombinantly produce the polypeptide of Beck et al. A person of ordinary skill in the art is motivated to make such kit and recombinantly produce the protein of Beck et al. because (1) a kit would allow all the required reagents to test enzymatic activity to be easily accessible, and (2) recombinant production of the protein would provide sufficient amounts of the protein for further characterization. One of ordinary skill in the art has a reasonable expectation of success at making the kit or recombinantly producing the protein because arranging all the required reagents in a kit and recombinant production of proteins is well known and widely used in the art. Therefore, the invention as a whole would have been prima facie obvious to a person of ordinary skill in the art at the time the invention was made.

***Conclusion***

39. No claim is in condition for allowance.

40. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PMR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

41. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Delia M. Ramirez, Ph.D., whose telephone number is (571) 272-0938. The examiner can normally be reached on Monday-Friday from 9:30:00 AM to 6:00 PM. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dr. Nashaat Nashed can be reached on (571) 272-0934. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (571) 272-1600.

/Delia M. Ramirez/

Delia M. Ramirez
Primary Patent Examiner
Art Unit 1652

DR
February 10, 2009